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Assessment of *In Vitro* Anti-melanoma Potential of *Ephedranthus pisocarpus* R.E.Fr.

LUBNA KARINE BESERRA SANTOS[1], MÁRCIA DENISE ALVES VERAS[2], KARINNE KELLY GADELHA MARQUES[3], MICHEL MUALÉM DE MORAES ALVES[1,4], ANDERSON NOGUEIRA MENDES[5], FERNANDO AÉCIO DE AMORIM CARVALHO[1], MARIANNA VIEIRA SOBRAL[3], MARIANA HELENA CHAVES[2] and JUAN CARLOS RAMOS GONÇALVES[1,3]

[1]*Medicinal Plants Research Center, Federal University of Piauí, Teresina, Brazil;*
[2]*Department of Chemistry, Federal University of Piauí, Teresina, Brazil;*
[3]*Department of Pharmaceutical Sciences, Federal University of Paraíba, João Pessoa, Brazil;*
[4]*Department of Veterinary Morphophysiology, Federal University of Piauí, Teresina, Brazil;*
[5]*Department of Biophysics and Physiology, Federal University of Piauí, Teresina, Brazil*

Abstract. *Background/Aim: Despite being a rare disease, melanoma is considered the most dangerous skin cancer due to its highly invasive and aggressive nature, and still requires for more effective treatments. The aim of this study was to evaluate the in vitro anti-melanoma potential of Ephedranthus pisocarpus R.E.Fr. (Annonaceae), a popular Brazilian plant with medicinal properties. Materials and Methods: Initially, the ethanolic extract (EtOH) was obtained from E. pisocarpus leaves and later partitioned using increasing polarity solvents. The anti-melanoma potential of E. pisocarpus was assessed by spectrophotometry and its cytotoxicity determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and confocal microscopy. Results: We demonstrated that the EtOH extract and fractions from E. pisocarpus had a moderate photoprotective action (FPS 3.0-5.0) against UVA radiation. Interestingly, the dichloromethane fraction presented higher anti-melanoma activity against B16-F10 (IC_{50}=46.8 μg/ml) and SK-MEL-28 cells (IC_{50}=40.1 μg/ml) and lesser toxicity on normal cells. Additionally, our study reported that spathulenol, one of the major constituents from E. pisocarpus, acts through an apoptosis-dependent mechanism in SK-MEL-28 cells. Conclusion: The present study demonstrated, for the first time, the in vitro anti-melanoma potential of E. pisocarpus against melanoma cells.*

Correspondence to: Juan C. R. Gonçalves, Ph.D., Department of Pharmaceutical Sciences, Federal University of Paraíba, Campus I, 58051-900, João Pessoa-PB, Brazil. Tel/Fax: +55 8332167347, e-mail: goncalvesjcr@ccs.ufpb.br

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Cutaneous melanoma is a type of skin cancer that originates in melanocytes and tends to show early metastasis secondary to the loss of cell adhesion in the primary tumor. Melanoma is considered one of the most aggressive cancer types and characterized by poor prognosis in its advanced stages (1, 2). Despite multiple conventional chemotherapeutic strategies for the treatment of melanoma, this condition remains one of the main fields of oncological research (3, 4). In fact, chemotherapy treatment is poorly effective, burdened with severe adverse effects, and still relies on relatively scarce effective options since melanoma cells are intrinsically resistant to treatments such as radiation and chemotherapy (5-8).

Natural products are one of the most important sources of potential anticancer drugs, representing more than 60% of clinically approved anticancer drugs used in therapeutics (9). Because of the diversity of their chemical structure and pharmacological mechanisms, the natural compounds are considered an alternative widely accepted in cancer therapy. However, since most anticancer drugs cause hematopoietic disorders and resistance to the chemotherapeutic regimen, drugs that have anti-metastatic efficacy and low toxicity in normal tissues are still necessary (10-12).

Ephedranthus is a neotropical genus that belongs to the Annonaceae family comprising six known species, found in Colombia and Paraguay, though most *Ephedranthus* species occur in the Amazon region (13-15). *Ephedranthus pisocarpus* R.E.Fr. is a large arboreal plant with medicinal properties popularly known as "envira-de-cocho" or “conduru” found in dry caatinga forests, cerrado, and the transition zone between cerrado and Amazonian rainforest in northeastern Brazil (13-15).

Since few studies have, thus far, investigated the pharmacologic properties of *E. pisocarpus* R.E.Fr. the aim of this study was to evaluate the *in vitro* anti-melanoma

potential of the ethanolic extract (EtOH) and fractions obtained from its leaves against melanoma and normal cells.

Materials and Methods

Chemicals and plant material. Ethanol, methanol, hexane, dichloromethane, ethyl acetate, doxorubicin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethylsulfoxide (DMSO), trypan blue, RPMI 1640, Dulbecco's Modified Eagle Medium (DMEM), trypsin/EDTA (0.25%), acridine orange and propidium iodide, were purchased from Sigma-Aldrich (San Luis, MO, USA). Fetal bovine serum (FBS) and penicillin/streptomycin were purchased from Thermo Fisher Scientific (□Waltham, MA, USA).

The leaves of *E. pisocarpus* R.E.Fr. were collected in the city of Jatobá do Piauí (S 04o 51' 47.1''; W 42o 03' 06.6'') in February 2012. Then, the specimen were deposited in the Herbarium Graziela Barroso (TEPB/UFPI, Teresina, PI, Brazil), with the registration number 16940.

In this study, all tested drugs were initially diluted in DMSO, forming a stock solution of 100 mg/ml that was kept at –20˚C, and further diluted to the desired concentrations immediately before the experiments. The total amount of DMSO used in all procedures did not exceed 0.5%.

Extraction and isolation of chemical constituents. The dried and ground leaves (1,560 g) of *E. pisocarpus* R.E.Fr. were subjected to maceration with ethanol for six times. The solutions obtained were pooled and the solvent removed on a rotary evaporator, yielding 160 g of ethanolic extract (EtOH). Then an aliquot of the EtOH extract (100 g, 100%) was solubilized in a MeOH/H_2O (2:1, v:v) mixture and partitioned using increasing polarity solvents such as hexane (Hex), dichloromethane (DCM), ethyl acetate (EtOAc) and aqueous (Aq) successively. The solutions were concentrated on rotary evaporator under reduced pressure, followed by lyophilization to obtain the following fractions: Hex (35.5 g, 35.5%), DCM (32.2 g, 32.2%), AcOEt (14.7 g, 14.7%) and Aq (13.4 g, 13.4%).

Determination of the solar photoprotective activity in vitro. The Solar Photoprotective Factor (SPF) was determined *in vitro* by the spectrophotometric method developed by Mansur *el al*. (16). For that, maximum absorbance values exhibited by the extract and fractions of *E. pisocarpus*, at concentrations ranging from 5 to 100 mg/ml, were determined by spectrophotometric scanning at 200-450 nm (PerkinElmer, Waltham, MA, USA), with 5 nm intervals using quartz cuvettes. Analyses were performed in triplicate and ethanol was used as blank (17, 18). Later, the SPF was calculated by the following equation:

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times SI\ (\lambda) \times Abs\ (\lambda)$$

where: EE (λ) is the erythemal effect spectrum for solar radiation; SI (λ) is the solar intensity spectrum; Abs (λ) represents the absorbance of sunscreen product and CF the correction factor (10). The EE x SI values are constants and have been determined by (19).

Animals. Male BALB/c mice were used in this study, animals were approximately 4 weeks old (25 g), maintained at the Medicinal Plants Research Center (NPPM/UFPI) *bioterium*, and kept in a controlled temperature (24±1˚C), 12 h light/dark cycle, with water/food *ad libitum*. All protocols were approved by the Animal Research Ethics Committee (CEEA/UFPI: 008/2012).

Cell cultures. B16-F10 (murine melanoma), SK-MEL-28 (human melanoma) and MRC-5 (normal human fibroblast) cell lines were acquired from Banco de Células do Rio de Janeiro (BCRJ, Rio de Janeiro, RJ, Brazil). Normal murine macrophages were collected from the peritoneal cavities of BALB/c mice as previously described (20). All cells used in this study were cultured in RPMI 1640 or DMEM, according to manufacturer, supplemented with 10% FBS, 100 IU/ml penicillin and 100 mg/ml streptomycin and placed in humidified air at 37˚C with 5% CO_2 atmosphere.

Hemolytic assay. To evaluate the hemolytic activity of *E. pisocarpus*, sheep red blood cells (RBCs) were collected with anticoagulant ethylenediamine tetraacetic acid (EDTA) and later diluted in PBS at 5% concentration. Then, 150 μl of EtOH, DCM, Hex, EtOAc and Aq were (50-500 μg/ml) and incubated for 1 h at 37˚C. After this period, the reaction was stopped by the addition of PBS (200 μl). The suspensions were then centrifuged at 1,000 rpm for 10 min at room temperature and the supernatant analyzed by spectrophotometry at 550 nm (BioTek, Winooski, VT, USA). The absence (negative control) and 100% hemolysis (positive control) were determined by replacing the sample solution tested with an equal volume of phosphate buffered saline (PBS) and sterile Milli-Q water, respectively (21).

MTT assay of cell viability. To assess the cytotoxic effects of *E. pisocarpus* EtOH extract and fractions (DCM, Hex, EtOAc, Aq) MTT assays were performed. Briefly, 5×10^4 cells were seeded in 96-well plates and incubated with the drugs at concentrations ranging from 0.5 to 500 μg/ml, for 24 h at 37˚C and 5% CO_2 atmosphere. Doxorubicin (5 μg/ml) was used as a positive control. Afterwards, 10 μl of MTT (5 mg/ml) were added and incubated for 4 h at the same conditions described above. Formazan crystals were dissolved in DMSO for additional 30 min, and the optical density recorded in a microplate spectrophotometer (BioTek) at 550 nm. Absorbance data were normalized to control (Treatment Absorbance/Control Absorbance×100) (9).

Determination of drug toxicity in macrophages. After being removed from BALB/c mice as described above murine macrophages (Mph) were plated in 96-well culture plates ($2x10^6$ cells/ml) and incubated at 37˚C and 5% of CO_2 during 3 h for cell adhesion. After the adhesion period, macrophages were incubated with *E. pisocarpus* EtOH extract and fractions (DCM, Hex, EtOAc, Aq) at concentrations ranging from 6 to 800 μg/ml, for 24 h. After this period, the cytotoxicity was determined by the MTT assay (20).

Quantification of apoptosis by acridine orange and propidium iodide. Melanoma cells (SK-MEL-28) were cultured at a density of 1×10^6 cells/ml in 24-well plates at 5% CO_2 and 37˚C conditions for 24 h. After that, spathulenol (50 μg/ml) was incubated for additional 24 h. The vehicle alone (DMSO 0.1%) and doxorrubicin (5 μg/ml) were used as negative and positive controls, respectively. Then, cells were harvested with trypsin/EDTA (0.25%), centrifuged (300 × *g*), washed with PBS and stained with 10 μl of acridine orange (AO) and propidium iodide (PI) solution (10 μg/ml). Freshly stained cells were observed under a confocal laser scanning microscope (Leica, Wetzlar, Hesse, Germany). The percentages of viable, early apoptotic, late apoptotic and secondary necrotic cells were determined in approximately 200 cells. The criteria for identification are as follows: (a) viable cells appear to have a light green nucleus and intact structure; (b) early apoptotic cells exhibit a bright-green nucleus showing chromatin condensation; (c) late apoptotic

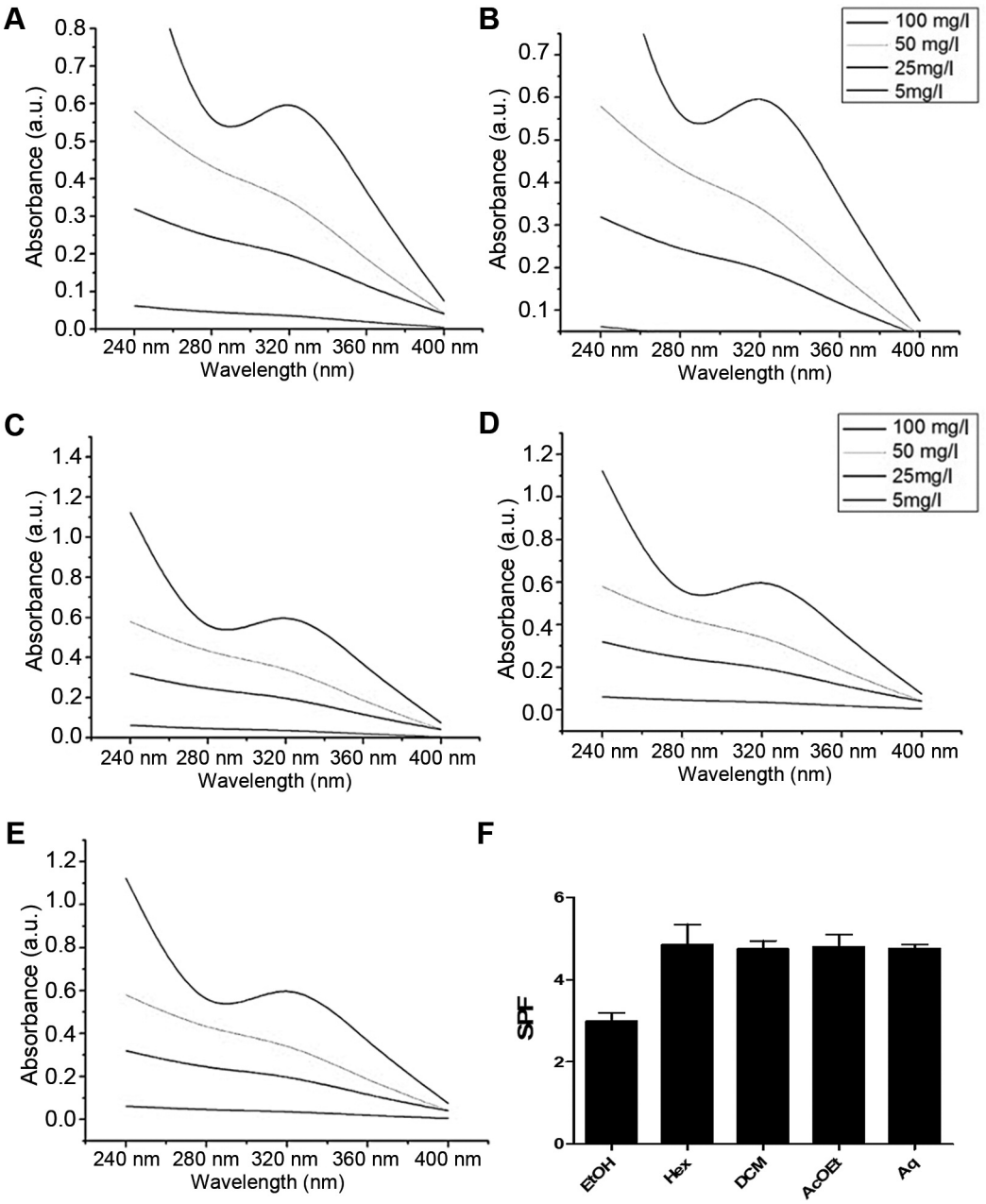

Figure 1. *Spectrophotometric absorption profile of E. pisocarpus EtOH extract (A) and fractions Hexane (B), DCM (C), AcOEt (D) and Aqueous (E). In (F) the Solar Protecion Factor (SPF) values calculated from samples are expressed at 100 mg/l. Data are represented as the mean±e.p.m. obtained from 3 independent experiments.*

cells show dense orange areas (green/red) of chromatin condensation and membrane blebbing; (d) secondary necrotic/dead cells appear to have red nucleus (12, 22, 23).

Statistical analyses. Statistical difference was assessed by one-way or two-way analysis of variance (ANOVA) followed by Dunnett's or Bonferroni's post-tests, as indicated below, and significance was considered when $p<0.05$. The half-maximal inhibitory concentrations (IC_{50}) were acquired by non-linear regression analysis (Graphpad Prism, San Diego, CA, USA).

Results

Photoprotective activity of E. pisocarpus R.E.Fr. Figure 1 shows the spectrophotometric absorption profile of the EtOH extract and Hex, DCM, EtOAC and Aq fractions

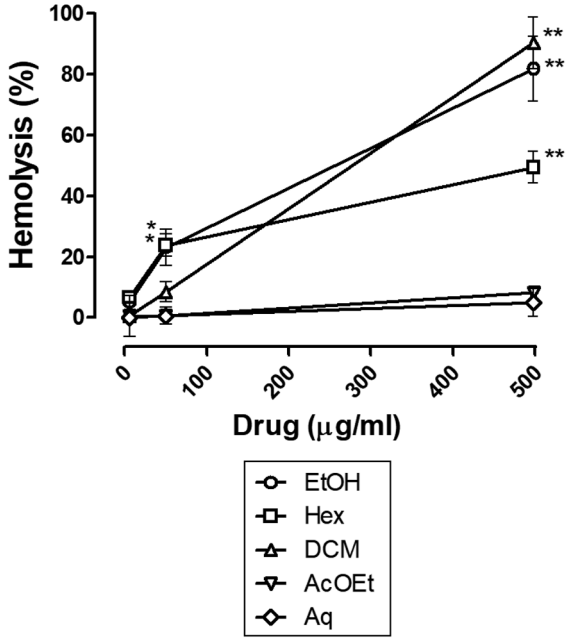

Figure 2. *Hemolytic activity of E. pisocarpus extract and fractions at concentrations ranging from 5 to 500 μg/ml. Data are represented as the mean±e.p.m. obtained from 3 independent experiments. ANOVA followed by Dunnet's test (*p<0.05; **p<0.01).*

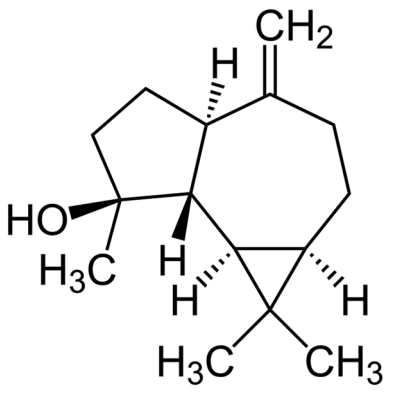

Figure 3. *Chemical structure of spathulenol.*

Table I. *Cytotoxic assessment of E. pisocarpus ethanolic extract and fractions against normal and melanoma cells.*

	IC_{50} (μg/ml)				
Cell type	EtOH	Hex	DCM	EtOAc	Aq
B16-F10	76.8	91.7	40.1	116.4	>1,000
SK-MEL-28	297.0	79.8	46.8	351.4	533.2
Mph	331.8	19.4	106.3	90.0	331.1
MRC-5	>1,000	>1,000	>1,000	>1.000	>1,000

EtOH: Ethanolic extract; Hex: hexane; DCM: dichloromethane; EtOAc: ethyl acetate; Aq: aqueous; IC_{50}: Drug concentration required to inhibit 50% of cell viability. Values are expressed by the mean of 3 independent experiments in triplicate.

from *E. pisocarpus*. Data show that all samples tested at 100 mg/l concentration induced light absorption in the region between 320 and 400 nm, characterized by the UVA radiation. Additionally, the SPF values exhibited by EtOH, Hex, DCM, EtOAC and Aq at 100 mg/l, were, respectively: 3.0±0.2; 4.85±0.5; 4.75±0.2; 4.80±0.3 and 4.76±0.1 (Figure 1F).

Evaluation of RBC hemolysis by E. pisocarpus R.E.Fr. The hemolytic assay evaluates the mechanical stability of the erythrocyte membrane as well as indicates damage mechanisms caused by various compounds during cytotoxicity screening. Several studies have reported that *in vitro* hemolysis assays have good correlations with *in vivo* toxicity by the hemolytic effect (24, 25).

In the present study, the hemolytic effect (%) of *E. pisocarpus* was observed by EtOH (23.0±5.9%) and Hex (23.7±3.7%), when incubated at 50 μg/ml ($p<0.05$). The DCM fraction exhibited toxicity to RBC only at the highest concentration tested (500 μg/ml) as demonstrated in Figure 2. No hemolytic activity was observed when AcOEt nor Aq were incubated at the same conditions (Figure 2).

Effects of E. pisocarpus on normal and melanoma cells. In order to investigate the anti-melanoma activity of *E. pisocarpus*, an MTT test (26) was performed using both human (SK-MEL-28) and mice (B16-F10) -derived melanoma cell lines. Additionally, drug toxicity was also evaluated on normal cells such as MRC-5 and mice peritoneal macrophage. The MTT method provides an indication of mitochondrial activity and cellular metabolic integrity and is commonly used to evaluate the cytotoxic effects of natural compounds (9, 27).

From the presented data, we were able to verify that the *E. pisocarpus* Hex and DCM fractions induced higher anti-melanoma effects, showing IC_{50} values of 79.8 μg/ml and 46.8 μg/ml on B16-F10 cells, respectively (Table I). DCM was also the more potent fraction against human melanoma cells (SK-MEL-28), exhibiting IC_{50} of 40.1 μg/ml (Table I). Although effectively against B16-F10 (IC_{50}=76.8 μg/ml), the EtOH extract from *E. pisocarpus* produced low activity on SK-MEL-28 (IC_{50}=297 μg/ml) (Table I). On the other hand, the EtOAc and Aq fractions practically did not induce cytotoxicity on both tested melanoma cell lines, exhibiting IC_{50} above 100 μg/ml (Table I).

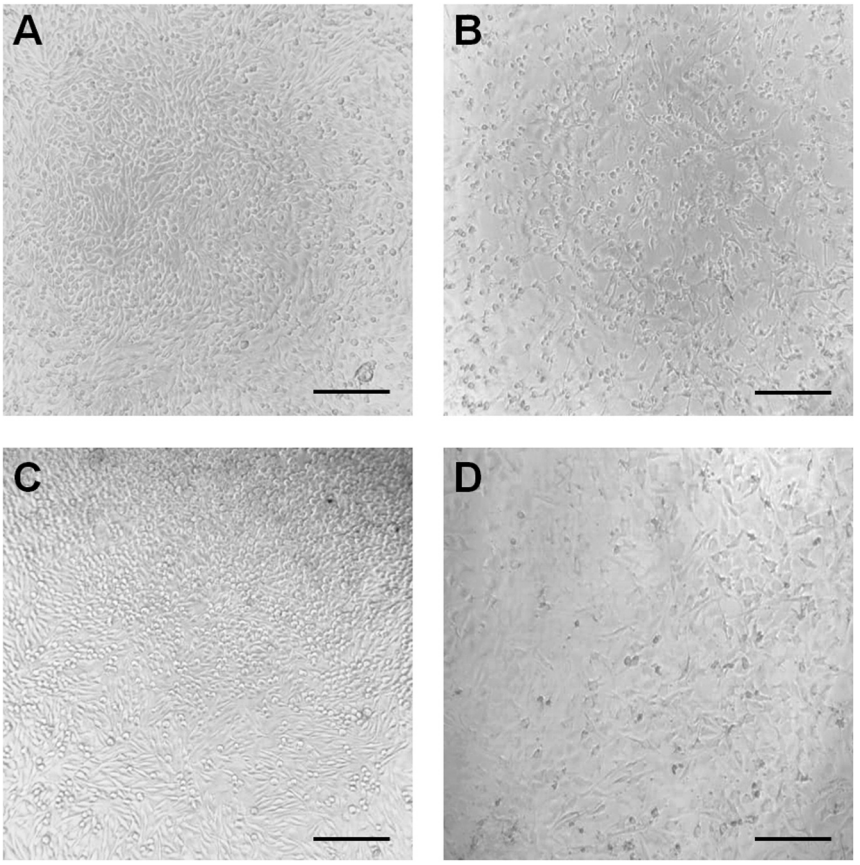

Figure 4. *Cytotoxicity of the major compounds obtained from E. pisocarpus against melanoma cells. The representative figures show melanoma (SK-MEL-28) cells after 24 h incubation with: (A) culture media alone (control); (B) doxorrubicin (5 μg/ml); (c) spathulenol (50 μg/ml); (d) 4α,10β-aromadendranodiol/1α-hidroxi-spathulenol mixture (50 μg/ml). Cells were observed at 100x magnification by phase-contrast microscopy (scale bar=100 μm).*

With exception of the Hex and EtOAc fractions, which showed toxicity on peritoneal murine macrophages (Mph) with IC_{50} of 19.4 μg/ml and 90.0 μg/ml, respectively (Table I), the other *E. pisocarpus* drugs tested (EtOH, DCM and Aq) showed lesser toxicity to these cell types. Interestingly, no toxic effect was observed against a normal human epithelial cell line (MRC-5) at the same conditions (Table I).

Cytotoxicity of the major constituents from E. pisocarpus. In another study conducted by our group (submitted for publication by Veras *et al*., 2019) we showed the phytochemical analysis of the Hex and DCM fractions obtained from the leaves of *E. pisocarpus*, where it was possible to isolate and identify various aromatic compounds, demonstrating the major presence of sesquiterpenes, highlighting the presence of spathulenol (SP), 4α,10β-aromadendranodiol and 1α-hydroxy-spathulenol, the latter two obtained as a mixture (AD/hSP).

Therefore, we evaluated whether spathulenol could be responsible for the observed anti-melanoma effect of *E. pisocarpus*. Additionally, we also investigated the activity of isolated 4α,10β-aromadendranodiol and 1α-hydroxy-spathulenol mixture (AD/hSP) against SK-MEL-28 cells. Based on Figure 4C, we observed that spathulenol (50 μg/ml) altered the fusiform cell morphology of SK-MEL-28 cells in control group (Figure 4A), acquiring a rounded shape and losing their ability to adhere in the plate. This effect was later confirmed by the MTT quantification assay (Figure 5), were spathulenol (50 μg/ml) reduced SK-MEL-28 viability from 100.2±7.8% (control) to 28.1±8.7% ($p<0.05$). AD/hSP also induced morphological changes into melanoma cells (Figure 4D), and also reduced cell viability to 33.8±8.2% ($p<0.05$) from control (Figure 5). As expected, the positive control doxorubicin (Doxo) reduced cell viability to 15.4±5.1% from control ($p<0.05$) (Figures 4B and 5). No significant effect was observed in the vehicle alone (DMSO 0.1%), when incubated under the same conditions (Figure 5).

Spathulenol induces apoptosis in melanoma cells. In order to evaluate the cytotoxic mechanism of spathulenol in SK-MEL-28 cells, we used the PI/AO double staining method.

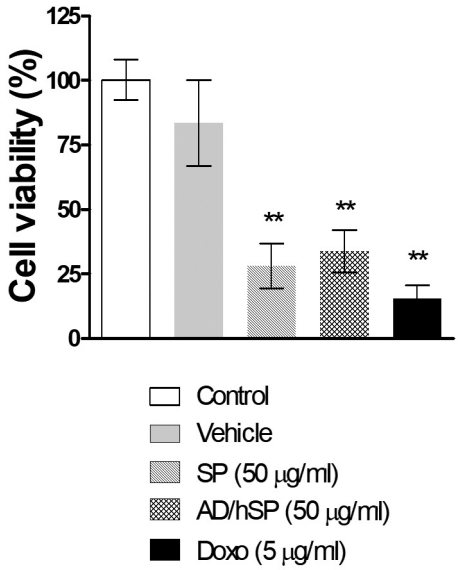

Figure 5. *Anti-melanoma activity of the major compounds obtained from the Hex and DCM fractions of E. pisocarpus, spathulenol (SP) and the mixture of 4α,10β-aromadendranodiol and 1α-hidroxi-spathulenol (AD/hSP). The vehicle was composed of DMSO 0.1% and culture media only. Doxorrubicin (Doxo) was used as positive control. Data are represented as the mean±e.p.m. obtained from 3 independent experiments. ANOVA followed by Dunnet's test (*p<0.05; **p<0.01).*

AO is a vital dye that can stain both live and dead cells, while PI stains only cells that have lost plasm membrane integrity. By laser confocal microscopy it was possible to analyze in detail the morphology and staining acquired by viable cells (light green), cells in early apoptosis (bright green) and late apoptosis (green/red) (12, 28).

Figure 6A shows that spathulenol (50 μg/ml) was able to increase the number of both PI and AO labeled human melanoma cells as well as apoptosis-derived morphological characteristics such as cell blebbing, chromatin condensation and nuclear fragmentation (arrows, Figure 6B). Posterior quantitative analysis depicted in Figure 7, revealed that the number of cells (%) in early apoptosis increased from 0.8±0.4% (control) to 69.4±3.9% by spathulenol ($p<0.05$). This sesquiterpene also increased late apoptosis cell counting from 1.7±1.2% (control) to 24.9±4.8% (Figure 7). As expected, doxorubicin at a concentration of 5 μg/ml was able to induce significant increase in both early and late apoptosis ($p<0.05$) (Figure 7). The vehicle alone was not able to induce any change in these parameters.

Discussion

Overexposure to radiation can cause premature aging, immunosuppression, chemical and histological changes in the epidermis, cataracts, and skin cancers like melanoma. It is estimated that 60-70% of cutaneous malignant melanomas are thought to be caused by ultraviolet (UV) radiation exposure (29). In order to prevent such radiation damage, the photoprotectors are indispensable tools (30). Sunscreens are photoprotective substances with the characteristics of absorbing, reflecting or refracting ultraviolet radiation, protecting the skin against direct exposure to sunlight (31, 32). Ultraviolet radiation is traditionally divided into: UVC (200-280 nm), UVB (280-320 nm) and UVA (321-400 nm) (31-33). Plant extracts and oils have been widely used in the cosmetics industry, mainly in the production of sunscreens, due to the photoprotective action of some species. However, it is important to emphasize that this activity depends among other factors on the capacity of UV radiation absorption and the chemical composition of the sample (17, 31).

The photoprotection activity observed in some plants is mainly due to the presence of certain classes of secondary metabolites such as flavonoids, coumarins, and alkaloids that protect the plant from the effects of UV radiation (18, 34). Given the lack of studies on the *Ephedrantus* species, we evaluated the photoprotective activity of *E. pisocarpus* R.E.Fr. *in vitro*. Our data demonstrated that *E. pisocarpus* R.E.Fr. exhibits moderate photoprotective activity, with FPS between 3.0 and 5.0, primarily from UVA radiation. This is an interesting finding, since UVA is much more abundant in sunlight (95%), penetrates more deeply into the dermis than UVB, and is involved in skin damage and tumorigenesis, inducing DNA damage by an oxidative stress-mechanism (29).

The hemolysis assay is considered an indispensable initial tool for screening toxicity reactions in red blood cells (RBCs) *in vivo* (35, 36). The term hemolysis refers to the disruption of RBCs, and this assay detects the leaking of intracellular contents including hemoglobin, which can be detected by spectrophotometry. In this study, it was demonstrated that the DCM fraction showed higher anti-melanoma potential *in vitro* among the *E. pisocarpus* tested drugs, since it presented highest cytotoxic potency against melanoma cells (B16-F10 and SK-MEL-28) and low toxicity on normal strains (RBC and Mph).

Spathulenol is a tricyclic sesquiterpenoid (Figure 3) found in several plants which has a role as a volatile oil component and plant metabolite. Although scarcely studied, it has been suggested that spathulenol has pharmacological effects such as anesthetic, vasodilator and suggested as the responsible compound for the cytotoxic effect of plants with anticancer activity (37-41). Although, very few studies have actually sought to evaluate the mechanism of action of this compound.

SK-MEL-28 is a melanoma cell line established from patient-derived tumor samples, initially obtained from an axillary lymph node of a 51-year-old male (42). In particular, SK-MEL-28 is a well stablished melanoma cell line, largely used for *in vitro* evaluation of anti-melanoma drugs and its

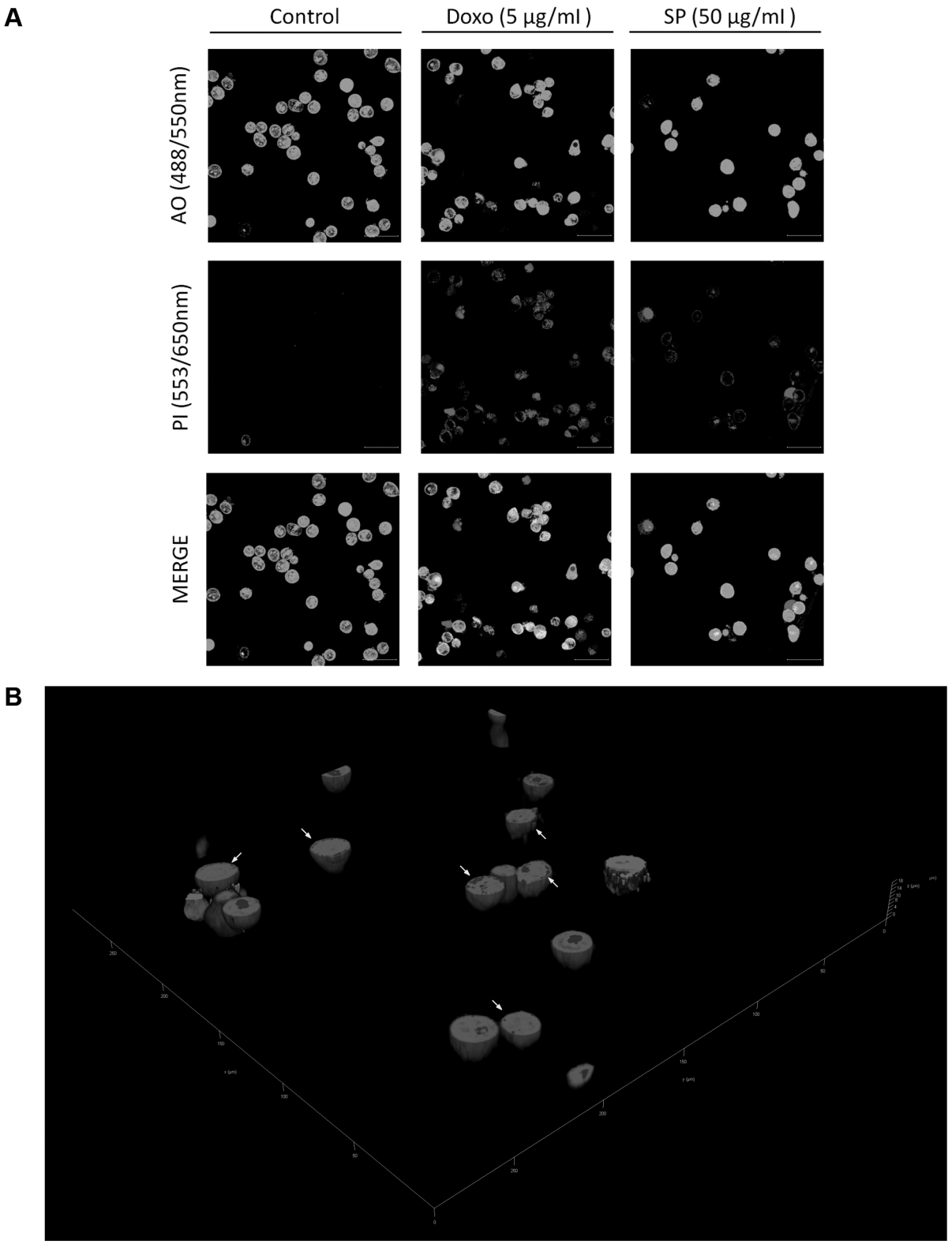

Figure 6. *Acridine orange (AO) and propidium iodide (PI) double staining for apoptosis evaluation. In A) are representative images of melanoma (SK-MEL-28) cells after 24h incubation with spathulenol (SP) isolated from E. pisocarpus. Doxorrubicin (Doxo) was used as positive control. B) 3D reconstruction of SK-MEL-28 cells after SP 50 µg/ml incubation. Confocal laser scanning microscopy under 400-630× magnification (scale bar=50 µm). Control cells were treated with culture media alone.*

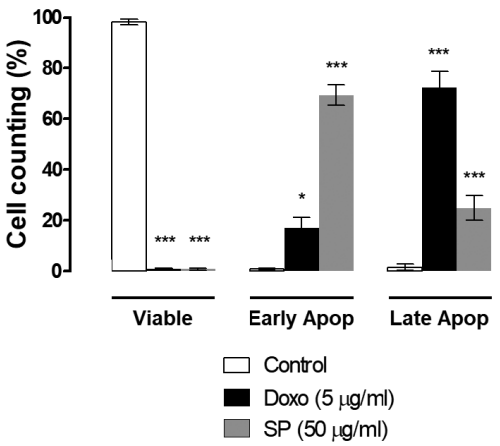

Figure 7. *Apoptotic melanoma cells after incubation with spathulenol (SP) isolated from E. pisocarpus. The graph displays the number of viable cells (%) at different apoptosis stages. Data are expressed as the mean±e.p.m. of at least 200 cells counted in 3 fields in duplicate. ANOVA two way followed by Bonferroni's post-test (**p<0.01; ***p<0.001).*

mechanisms. This cell line expresses mutant B-Raf (BRAF) and wildtype N-Ras, also being able to form tumors in nude mice (43).

Apoptosis is known as the process of programmed cell death characterized by distinct morphological characteristics and energy-dependent biochemical mechanisms (44). The understanding of cellular alterations during apoptosis as well as its molecular mechanisms has provided the basis for novel targeted therapies to treat cancer. In fact, apoptosis induction is considered an efficient strategy to prospective studies involving new anticancer drugs (9, 45).

The treatment of melanoma with the current drugs available in clinics still has many difficulties. This is because melanoma cells possess aggressive nature and high resistance to standard drugs like dacarbazine. Recent studies have suggested the importance of the proto-oncogene BRAF V600E mutation, as a hallmark for high-risk melanoma associated with shortened patient survival rates and tumor drug resistance (46, 47). The BRAF mutation in SK-MEL-28 cells results in constitutive activation of the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) cascade, which causes uncontrolled cell growth (46, 47).

Our study demonstrated for the first time that cell death promoted by spathulenol occurs via an apoptosis mechanism in human melanoma cells (SK-MEL-28) and might be responsible for the *in vitro* anti-melanoma effect promoted by the DCM fraction obtained from *E. pisocarpus*. This hypothesis is corroborated by other studies, such as that of Ziaei *et al*. (48) which demonstrated that spathulenol possess the capacity to inhibit lymphocyte proliferation by inducing apoptosis through a caspase-3 independent pathway. This mechanism may also involve BRAF inhibition and/or the MAPK pathway in SK-MEL-28 cells. However, further studies are needed to describe in detail the mechanisms involving the cytotoxicity of spathulenol in melanoma cells.

Conclusion

Taken together, our results demonstrated the *in vitro* photoprotective and antimelanoma potential of the EtOH extract and derived fractions (Hex, DCM, AcOEt and Aq) isolated from the leaves of *E. pisocarpus*. When evaluated against normal cells such as RBC, macrophage and human fibroblast, it was evidenced the lower toxicity presented by the DCM fraction. Additionally, our study reported the cytotoxic action of the sesquiterpene spathulenol, one of the major constituents of *E. pisocarpus*, which acts by an apoptosis-dependent mechanism. These findings will certainly be of great value for future studies *in vivo* aiming to demonstrate the anti-melanoma activity of *E. pisocarpus* derived-drugs and its constituents, contributing to the development of more effective drugs for the treatment of skin cancer. Therefore, the present study demonstrated for the first time, the anti-melanoma potential *in vitro* of *E. pisocarpus* R.E.Fr. against melanoma cells.

Conflicts of Interest

The Authors declare no conflicts of interest in this work.

Authors' Contributions

Lubna K. B. Santos, Márcia D. A. Veras, Karinne K. G. Marques and Michel M. M. Alves performed all the experiments, analyzed the results and wrote the article. Mariana H. Chaves supervised the obtention of the test compounds and edited the article. Anderson N. Mendes and Fernando A. A. Carvalho supervised the toxicity study in normal cells and edited the article. Marianna V. Sobral and Juan C. R. Gonçalves supervised the antimelanoma study and edited the article. Juan C. R. Gonçalves revised and approved the final article.

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